

Amendments to the Specification:

Please replace the paragraph at page 2, line 31 to page 3, line 3, with the following amended paragraph:

In another aspect, the invention relates to an antibody which binds specifically to the protein of the invention and any other antibody that competes directly or by steric stearic hindrance therewith for said protein. Typically, the antibody is a monoclonal antibody. In one embodiment, the antibody is a class M immunoglobulin with a kappa-light chain.

Please replace the paragraph at page 6, line 30 to page 7, line 8, with the following amended paragraph:

The monoclonal antibodies of the invention may be the complete antibodies described herein, or fragments thereof. That is, they may be any fragment of a monoclonal antibody of the invention that specifically recognises the protein of the invention. Such fragments include Fab, F (ab') 2, Fab', etc. These fragments ~~can~~ can be prepared by digestion with an enzyme such as papain, pepsin, ficin, or the like. The properties of the obtained fragments can be confirmed in the same manner as described herein.

Please replace the paragraph at page 9, lines 20-32 with the following amended paragraph:

Fig 2 shows a ~~comparision~~ comparison of MQ1 expression, by immunocytochemistry and flow cytometry, on a range of fetal astrocyte cultures and astrocytoma cell lines. A-C show immunocytochemistry on live cells of a grade IV, grade III and 16 week gestation fetal astrocytes respectively. D-F show the corresponding flow analysis with the same cells with the level of MQ1 surface expression estimated as mean channel fluorescence. G D shows the results

of the flow analysis plotted as a graph. This demonstrates an inverse correlation of cell surface MQ1 protein expression with fetal astrocyte maturity and correlation with astrocytoma grade;

Please replace the paragraph at page 10, lines 20-26, with the following amended paragraph:

Fig. 6 shows ~~MQ1 immunocytochemistry~~ of G-CCM cells treated with (A) 0.1 μ m control oligo, (B) 0.5pm control oligo (C) 1.0 μ m control oligo, (D) 0.1 μ m anti-sense MQ1 oligo, (E) 0.5 μ m anti-sense MQ1 oligo and (F) 1.0 μ m antisense oligo, showing that ~~MQ1 anti-sense oligo knocks out MQ1 protein expression at concentrations of 0.5 and 1.0 pm~~;

Please replace the paragraph at page 10, lines 31-32, with the following amended paragraph:

Fig. 8 shows immunocytochemistry (ICC) detection of cleaved Caspase 3 A (control) following oligo treatment B ; and

Please replace the paragraph at page 11, lines 17-27, with the following amended paragraph:

All cell culture reagents were obtained from Gibco BRL (Paisley, UK) with the exception of the hypoxanthine, aminopterin and thymidine (HAT) and the hypoxanthine and thymidine (HT) that were obtained from Sigma (Poole, Dorset, UK). The secondary and negative control antibodies were supplied by Dako (Bucks, UK). The PARP and Caspase3 antibodies were purchased from Sigma (Poole, Dorset, UK) and the Protein-A Sephadex SEPHAROSE CL4B from Pharmacia Biotech (Herts, UK). PTO linked oligonucleotides were obtained from MWG-Biotech (Germany).

Please replace the paragraph at page 12, line 30 – page 13, line 25, with the following amended paragraph:

Mabs were produced utilizing a standardized protocol designed to promote a rapid predominantly IgG response. In brief, a BALB/c mouse was inoculated intra-peritoneally with 5x10⁶ G-CCM cells in 1ml of Freund's complete adjuvant. Similar doses emulsified in Freund's incomplete adjuvant were administrated 14 and 28 days later to boost the immune response. Four days after the final booster inoculation the mouse was killed, its spleen aseptically removed and the splenocytes induced to fuse with NSO myeloma cells (at a ratio 5:1) using polyethylene glycol. The resulting fusion products were suspended in a selective, HAT-supplemented, growth medium (RPMI-1640 medium containing 10mM L-glutamine, 1% sodium pyruvate, 100 iu/ml penicillin, 100 μ g/ml 100 μ g/ml streptomycin and 20% MYOCLONE Myeclone FCS) and seeded into 96-well plates. The medium, from the viable hybridomas produced, was screened by indirect immunofluorescence against live and acetone-fixed G- CCM cells. Those showing specific recognition were recloned three times, to ensure monospecificity, in HT-supplemented growth medium and stored in liquid nitrogen. The hybridoma cell line MQ-1, which produced an antibody recognizing a cell surface antigen was propagated as an ascitic tumour in BALB/c mice previously immunosuppressed with Pristane. The ascitic fluids were collected, centrifuged and frozen at -20oC until use.

Please replace the paragraph at page 14, lines 3-18, with the following amended paragraph:

Hybridoma medium (neat) or ascites fluid (diluted 1: 200 in PBS) was incubated with living cells, grown to 90% confluence on coverslips, for 40 min at room temperature (RT). After washing, the cells were fixed in acetone at -20oC for 10 min followed by rehydration in PBS and incubation with an FITC- conjugated rabbit antimouse antibody (FITC-RAM) for 30 min at RT. After two further washes the cells were mounted on a glass slide, in a drop of Citifluor CITIFLUOR, and examined using a Zeiss immunofluorescence microscope or a Biorad confocal

microscope. Incubations in PBS without primary antibody were used as negative controls. The fluorescent labelling of positive cells was subjectively rated from low intensity (+) to high intensity (+++).

Please replace the paragraph at page 14, line 28 – page 15, line 14, with the following amended paragraph:

Cultured cells were removed from the flasks by trypsinization, counted and aliquoted into centrifuge tubes at a concentration of 5x10⁵ cells per tube. Triplicate samples were incubated in excess ascitic fluid in 200 \pm 1 200 μ l of serum free medium containing 1% bovine serum albumin (SFM/BSA) for 40 min at RT with gentle agitation. Following 2 washes in SFM the cells were incubated in an FITC- RAM antibody for 30 min at RT with gentle agitation. The cells were then washed twice in SFM and fixed in PBS containing 1% para-formaldehyde. The samples were analysed within 48 hr of fixation, using a Coulter EPICS Elite ELITE flow cytometer. Negative controls were incubated with an antibody raised against *Aspergillus niger* glucose oxidase, an enzyme not present or inducible in mammalian cells. The consistency of the mean channel fluorescence measurements between sample batches was checked using EPICS Immuno-Brite standards.

Please replace the paragraph at page 15, line 18 – page 16, line 24, with the following amended paragraph:

On receipt the tissue was fixed in 10% formalin prior to routine embedding in paraffin wax using a ~~Tissue Tex~~ TISSUE TEX VIP (Miles Scientific) automated processor. The paraffin blocks were sectioned at a thickness of 6mm and mounted onto 3- aminopropyltriethoxysilane-coated slides. The tissue sections for indirect immunohistochemistry were processed using an avidin-biotin peroxidase complex (ABC) method. The tissue was dewaxed in xylene and rehydrated before endogenous peroxidase activity was blocked by a 10min incubation in 3% H₂O₂ in methanol at room temperature (RT). To counter antigen masking, due to the formalin fixation, the tissue was pretreated with microwave irradiation to promote antigen retrieval. The sections

were washed in distilled water and placed in 0.01M Tri-Na citrate pH7.8 and irradiated in a Miele microwave oven for 6min (2x3min) at 450W (the optimal time and intensity of irradiation was determined from preliminary studies). After incubation in PBS containing 5% normal rabbit serum for 10min at RT the sections were incubated in MQ1 ascites (diluted 1: 50 in PBS) at 4C overnight. Following 2x5min washes in PBS the sections were incubated in biotinylated rabbit anti-mouse IgM diluted 1: 400 in PBS for 40min at RT. After further washes in PBS, a streptavidin-biotin complex linked to peroxidase was added to the sections and incubated for 40min at RT. The peroxidase reaction was developed in 0. 1% diaminobenzidine in PBS activated with 1% H202. After washing in water, the sections were counterstained in haematoxylin, dehydrated through graded alcohols, cleared in xylene and mounted in DPX. In addition to negative controls, incubated with a primary antibody raised against Aspergillus niger glucose oxidase, positive controls of histologically normal brain and astrocytoma tissue were included with every batch. cDNA Expression Library and screening.

Please replace the paragraph at page 16, lines 26-31, with the following amended paragraph:

G-CCM Cell cDNA Library Synthesis

A Total RNA Isolation from G CCM cells

This was performed using Tel-Test TEL-TEST RNA Stat-60, following their guidelines. Web Site [www\[dot\]isotexdiagnostics\[dot\]com/rna_stat-60_reagent\[dot\]html](http://www.isotexdiagnostics.com/rna_stat-60_reagent.html)
[www<dot>isotexdiagnostics<dot>com/rna_stat-60_reagent<dot>html](http://www.isotexdiagnostics.com/rna_stat-60_reagent.html)

Please replace the paragraph at page 17, lines 1-5, with the following amended paragraph:

B mRNA Purification from Total RNA

This was performed using Invitrogen's FastTrack FASTTRACK 2.0 Kit, following their guidelines. Web Site [www\[dot\]invitrogen\[dot\]com/content\[dot\]cfm?pageid=3443&efid=3308](http://www.invitrogen.com/content/cfm?pageid=3443&efid=3308)

35&cftoken=53475959#FastTrack

[wwwinvitrogencom/contentcfm?pageid=3443&cfid=3308](http://www.invitrogen.com/content/cfm?pageid=3443&cfid=3308)

35&cftoken=53475959#FastTrack

Please replace the paragraph at page 17, lines 7-12, with the following amended paragraph:

C cDNA Library Synthesis from mRNA

This was performed using a Stratagene cDNA synthesis kit (following their protocol).

Stratagene ZAP Express EXPRESS cDNA Synthesis Kit Instruction Manual

[wwwstratagenecom/manuals/200403pdf](http://www.stratagene.com/manuals/200403.pdf)

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Please replace the paragraph at page 23, lines 8-14, with the following amended paragraph:

Of the primary breast tumours tested 137/228 were MQ1 positive while fibrocystic disease and fibroadenoma tissues, both premalignant conditions displayed no MQ1 positivity. Figures Figure 5 shows strong MQ1 positivity in invasive ductal carcinoma cells and lobular carcinoma cells surrounded by MQ1 negative stroma.

Please replace the paragraph at page 24, line 2 – page 25, line 17, with the following amended paragraph:

The PTO linked antisense oligonucleotide was designed against the transcription initiation site and kozac sequence at the beginning of the Jagged1 gene (Accession number AF028593). The control oligonucleotide oligonucleotide was the same 18 mer with one base changed (therefore being the tightest control possible to generate). Both oligonucleotides were synthesized by MWG Biotech. For colony count assays G-CCM cells were seeded out into 24well 24-well

plates at 50,000 cells/well. The cells were incubated for 24hrs in growth medium and then washed with serum free medium (SFM). The cells were then either treated with lipofectin LIPOFECTIN (Invitrogen Life Technologies) alone following the standard protocol (at 5 μ l/ml) or lipofectin LIPOFECTIN with the antisense and antisense control oligonucleotides at a range of concentrations (0.1, 0.5 and 1.0 μ M) for 16hrs. Following treatment the cells were washed twice with SFM and then incubated in growth medium for 24 and 48hrs. The results (Figure 6) show that treatment with the antisense oligonucleotide at concentrations of 0.5 and 1.0 μ M reduced the tumour cell population when compared to the control oligonucleotide and lipofectin alone treatment. To assess whether this was due to the induction of apoptosis similarly treated cells were harvested for their protein and examined for Parp cleavage (an indicator of apoptosis) by immunoblotting. The results (Figure 7) clearly show a reduction in the level of Parp at 0.5 and 1.0 μ M antisense oligonucleotide treatment when compared to control oligonucleotide and lipofectin alone treatment. Thus indicating that the antisense oligonucleotide treatment induces apoptosis in the G-CCM cells. To confirm this, treated G-CCM cells were also examined for the presence of cleaved Caspase 3 (another indicator of apoptosis) by immunocytochemistry. The results (Figure 8) show that G-CCM cells treated with 1.0 μ M displayed caspase 3 cleavage thus indicating that apoptosis was being induced. To demonstrate that these effects were due to the knocking out of the MQ1 proteins by the antisense oligonucleotides, treated cells were examined for the presence of the MQ1 proteins by immunocytochemistry with the MQ1 antibody. The results (Figure 9) show that the expression levels of the MQ1 proteins is reduced by antisense oligonucleotide treatment when compared to the control oligonucleotide.